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Propagule pressure helps overcome adverse environmental conditions during population establishment

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ABSTRACT

The establishment success of a population is a function of abiotic and biotic factors and introduction dynamics. Understanding how these factors interact has direct consequences for understanding and managing biological invasions and for applied ecology more generally. Here we use a mesocosm approach to explore how the size of founding populations and the number of introduction events interact with environmental conditions (temperature) to determine the establishment success of laboratory-reared *Drosophila melanogaster*. We found that temperature played the biggest role in establishment success, eclipsing the role of the other experimental factors when viewed overall. Under optimal temperature conditions propagule pressure was of negligible importance to establishment success. At adverse temperatures, however, establishment success increased with the total founding population size. This effect was considerably stronger at the cold than at the hot extreme. Whether the population was introduced all at once or by increments (changing the number of introduction events) had a negligible global effect. However, once again, a stronger effect of increasing number of introduction events was seen at adverse temperatures, with hot and cold extremes revealing opposite effects: adding flies incrementally decreased their establishment success at the hot extreme, but increased it at the cold extreme. These differing effects at hot and cold thermal extremes implies that different establishment mechanisms are at play at either extreme. These results suggest that the effort required to prevent (or conversely, to facilitate) the establishment of populations varies with the environment in ways that can be complicated but predictable.

1. Introduction

An understanding of the factors involved in the successful establishment of populations is critical to understanding how species shift their ranges, with direct applied implications for reintroduction biology, invasion science and assisted migration (Hayes and Barry, 2008; Emiljanowicz et al., 2017; Kueffer et al., 2013). In general, establishment success is a function of environmental suitability, life history traits and introduction dynamics (Howeth et al., 2016; Iles et al., 2016; Casties et al., 2016; Wilson et al., 2009; Cassey et al., 2004; Enders et al., 2020; Catford et al., 2009), with important factors including the recipient environment's climate, biodiversity and disturbance regime; the reproductive capacity and adaptability of the introduced species; the number of individuals introduced; and the pathway along which individuals are introduced. The interplay of these factors is often context-specific, varying in space and time. However, across taxa, the most consistent predictor of establishment success is the total number of individuals introduced, termed propagule pressure (Lockwood et al., 2005; Lockwood et al., 2009; Simberloff, 2009; Cassey et al., 2018).

Propagule pressure, which is essentially founding population size, can be important in establishment success for both demographic and genetic reasons (Fauvergue et al., 2012; Hufbauer et al., 2013; Drake and Lodge, 2006). Small founding populations are more likely to die out simply by chance, e.g. all individuals born are one sex or all happen to succumb to adverse environmental conditions (Fauvergue et al., 2012; Duncan et al., 2014). Moreover, the per capita fitness might be intrinsically lower at low population sizes, i.e. Allee effects. Propagule pressure can also have genetic effects—small founding populations will typically have low genetic diversity and so over time they can become heavily inbred. High levels of inbreeding can be detrimental to survival (Fauvergue et al., 2012; Szűcs et al., 2014), although in some cases it can also be advantageous, e.g. low genetic diversity can reduce intra-specific

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competition and facilitate invasions in eusocial insects (Tsutsui et al., 2003; Garnas et al., 2016).

Environmental stochasticity can similarly interact with factors intrinsic to small populations to increase the chance that a population dies out (Duncan et al., 2014; Bajeux et al., 2019). The interaction between founding population size and environment is complex and the interplay is context-specific (Duncan et al., 2014; Szűcs et al., 2014; Duncan, 2016; Bacon et al., 2014; Dressler et al., 2019; Von Holle and Simberloff, 2005; Chevillon et al., 2013), making it difficult to elucidate which mechanisms are important in specific cases.

When viewed across the whole range of environmental suitability and propagule pressure, both population size and environment play a role (Duncan, 2016; Yeates et al., 2012; Hee et al., 2000) and therefore, as a result of environmental stochasticity, both the total number of individuals introduced and when they are introduced in relation to each other can impact establishment success (Dressler et al., 2019; Von Holle and Simberloff, 2005; Vahsen et al., 2018; Hedge et al., 2012; Sinclair and Arnott, 2016).

The interaction between propagule pressure and environmental stochasticity has direct implications for managing biological invasions. For example, biotic and abiotic factors and introduction dynamics need to be considered before deciding how many individuals of a biocontrol agent need to be released for a population to establish (Fauvergue et al., 2012; Hufbauer et al., 2013; Duncan, 2016; Yeates et al., 2012). The influence of introduction events on population establishment is similarly important when trying to prevent invasions (i.e. biosecurity) (Garnas et al., 2016; Wonham et al., 2013). Is it enough to keep each introduction event below a certain establishment threshold? Or is the total number of individuals introduced more important, regardless of whether they arrive during a single incursion event or are distributed over many? Are there particular sets of environmental conditions when establishment is much more likely (i.e. invasion windows)? Despite the practical importance of these questions, little consensus on the relative role of these factors has been reached to date in the literature (Cassey et al., 2018; Wonham et al., 2013). It might be expected that multiple introductions should increase the probability of population establishment and survival in variable, adverse or harsh environments, as the timing of at least one introduction is more likely to coincide with suitable conditions for establishment (Haccou and Iwasa, 1996; Drake et al., 2006) (but see also Koontz et al., 2018). One might similarly expect that if strong Allee effects are present, small introductions will have a low probability of survival and a single large introduction event would be more likely to result in successful establishment (Szűcs et al., 2014; Hedge et al., 2014). However, various studies have found number of individuals introduced (Vahsen et al., 2018; Sinclair and Arnott, 2016), number of introduction events (Hedge et al., 2012; Sinclair and Arnott, 2016; Koontz et al., 2018), both (Dressler et al., 2019; Drake et al., 2005) or neither (Sinclair and Arnott, 2017) to influence the probability of establishment. Similarly Garnas et al. (2016) note that founder effects rarely limit invasive insect fitness. This is most likely due to different mechanisms at play in different taxa or environments (Drake and Lodge, 2006; Vahsen et al., 2018).

Laboratory studies are useful in addressing such questions, since there is typically tighter control over key demographic factors, with fewer uncontrolled covariates than under field conditions (Dressler et al., 2019). As ectotherms, most insects have body temperatures that approximate closely (and are therefore susceptible to) ambient environmental conditions (Sinclair et al., 2016). Furthermore, insects typically show asymmetric thermal performance curves of diverse traits that manifest as a peak in performance (often equated with fitness or population growth rates) at some intermediate temperature, while performance declines to zero at extremes, albeit far more sharply at high temperatures (Buckley et al., 2017; Frazier et al., 2006). *Drosophila melanogaster* (Diptera: Drosophilidae) is a well-studied model system in biomedical research and evolution owing to its short life-span, well-characterised genetics and ease of handling and is increasingly recognised as a model in invasion biology (Roberts, 2006; Gibert et al., 2016; Dahmann, 2008). Studies on *D. melanogaster* have provided insight both to the invasion of other drosophilid flies, such as *D. suzukii* (Hauser, 2011; Iacovone et al., 2015; Asplen et al., 2015) and to biological invasions more generally (Gibert et al., 2016).

In this study, we investigated the interplay between environment, founding population size and number of introduction events on establishment success of *D. melanogaster* laboratory colonies (with establishment success defined here as when offspring of the founding individuals survived to adulthood (Szűcs et al., 2014)). First, from thermal performance curve theory we predicted that temperature was likely to have the greatest effect on establishment, with a higher proportion of colonies establishing at optimal temperatures than at either thermal extreme. Second, although *Drosophila melanogaster* is able to establish from a single gravid female under optimal conditions, laboratory experiments often initiate multiple introductions to compensate for anticipated colony failure under these circumstances. Therefore we expected that increasing founding population size would increase the probability of establishment under all circumstances, with a greater effect at extreme thermal conditions since, under those conditions, small founding populations may fail more often. Third, previous studies have shown that in sexually reproducing organisms, colonies established from a single large founding event had higher survival rates than those from smaller and more frequent introductions (Sinclair and Arnott, 2016). Thus, we predicted that increasing the number of introduction events (which in turn decreased the number of individuals in each event) would decrease the probability of successful colony establishment. Finally, we predicted that these factors would interact positively with each other to influence overall colony establishment, such that colonies established at optimum temperatures with the highest number of founders in a single event would be most likely to survive.

2. Methods

2.1. *Drosophila melanogaster* colonies

Drosophilid flies were wild-caught in Stellenbosch, South Africa by trapping with mixed rotting fruit. Fourteen gravid females were placed individually into vials containing a media diet (described below). Once larvae were observed, the female was removed and identified by sequencing of the COI barcode gene (Folmer et al., 1994) and compared to reference sequences on Genbank (Benson et al., 2005). All females identified unambiguously as *D. melanogaster* and offspring were then mixed in colonies.

Colonies were maintained on a media diet consisting of 15.875 g brewer's yeast, 9.175 g soy flour, 70 g corn flour, 21.175 g dextrose, 6 g agar, 15 mL of a 3% nipagin / ethanol solution and 5 mL of a 41.8% propionic / 4.2% phosphoric acid solution, mixed with enough water to make 1 L. The mixture, excluding nipagin and acid, was heated until it thickened, then nipagin and acid were added and the mixture stirred until it cooled. It was then poured into sterile glass containers and allowed to solidify overnight. Plain paper was added to provide adult flies a resting place and allow for larvae to crawl up and pupate, after which containers were plugged with cotton wool or covered with fine mesh (depending on the width of the container's opening). Optimal rearing temperature for *Drosophila melanogaster* colonies is between approximately 18 °C and 25 °C (Stocker and Gallant, 2008). We chose to maintain our colonies at 22 ± 1 °C at a 14h:10 h L:D cycle and 45–55% humidity, as the fly developmental time at this temperature allowed us greater control over the colony. It was necessary to periodically transfer flies to new containers with new media as the diet media became depleted and quality deteriorated. In doing this, not all adult flies could be transferred and thus populations were routinely culled in the transfer process.

The genetic diversity of the wild-caught flies was unknown. However, we were careful to mix offspring and colonies during transfers, ensuring as much genetic mixture as possible. Due to the colony being started from 14 females, maintained in the laboratory for multiple generations and routinely culled (which could cause a population bottleneck) it is unlikely that there were large genetic differences between flies used in the experiments.

2.2. Experimental design

A pilot study was carried out to determine the temperatures at which 40–60% of founding colonies of eight females and eight males would establish. Treatments were conducted at 12 °C, 14 °C, 16 °C, 28 °C, 30 °C, 32 °C and 33 °C, each replicated 20 times. Adult flies of 1–4 days old from the mass-bred fly cultures were caught and cooled on ice and then sexed. While cool they were sucked into plastic tubing and allowed to regain mobility before being gently blown into the treatment containers. All introductions were performed between 7:00 and 9:00am to control for potential circadian effects on reproduction. Given the age of flies, most females were expected to be gravid, although this was not explicitly determined. Founding adult flies were removed at approximately two thirds of the fly life cycle so as not to be confused with the establishment cohort. *Drosophila melanogaster* life cycle from egg to adult was *c*. 52, 12 and 9 days, at 13 °C, 22 °C and 32 °C, respectively. Establishment was defined as offspring surviving to adulthood, which we considered to be the case if at least one adult fly emerged and survived for at least two days. *Drosophila melanogaster* mate approximately one day after emergence, thus allowing two days for survival implied that (if the environment were maintained) the emergent flies would be able to mate and reproduce, leading to successful establishment of multiple generations.

From the pilot study, thermal extremes were determined to be 13 °C and 32 °C. Optimal temperature was kept at 22 °C, as this was the temperature at which the colonies were reared. All treatments were therefore conducted at either 13 °C (extreme cold), 22 °C (optimal) or 32 °C (extreme heat). All treatments were conducted in programmable, insulated growth chambers equipped with fluorescent lights. Temperatures were kept within 1 °C of the intended target temperatures throughout the course of the experiment (as monitored by the use of digital thermometers). A partially factorial experimental design was applied to test the effect of temperature (13 °C, 22 °C or 32 °C), founding population size (2, 4, 8 or 16 individuals) and number of introduction events (1, 2, 4 or 8 events), as shown in Fig. 1. For instance, a founding population size of 16 with 8 introduction events meant that two new adult flies were introduced to the experimental container every day eight times (Fig. 1). To prevent crowding (Horváth and Kalinka, 2016; Miller and Thomas, 1958), treatment container size and diet media amount were matched to founding population size. Containers were wide-mouthed glass jars of 1 L, 500 mL, 250 mL and 125 mL with 160 mL, 80 mL, 40 mL and 20 mL diet media for founding population sizes of 16, 8, 4 and 2 flies (1:1 female:male ratio), respectively. While this potentially introduces a confounding effect of jar size, the alternative of potentially introducing crowding effects was deemed less desirable as crowding can have large negative impacts on development and survival (Horváth and Kalinka, 2016). Fresh media was made and added a day before treatments began. Adult flies were caught, sexed and added to treatment jars using the same method as for the pilot study. Adult flies were kept in the treatment containers for approximately two thirds of the fly life cycle at that respective temperature, as identified in the pilot study, and then removed. Containers were subsequently monitored every day for emergence of new adult flies. Monitoring continued for five days after the first new adult had emerged at 32 °C and 22 °C and for 10 days afterwards at 13 °C. This resulted in an experimental duration of 12–14, 15–17 and 56–60 days for treatments at 32 °C, 22 °C and 13 °C, respectively. Ten replicates were performed at each treatment condition, resulting in a total of 300 replicates. Due to space and time constraints, not all treatments could be conducted simultaneously. Treatments were carried out in batches of between two and six treatments (i.e. 20 to 60 replicates) started on the same day, with media prepared in a single batch. Treatments were randomly assigned to each batch, modified slightly when necessary due to space constraints, resulting in seven treatment batches throughout the course of the experiment.

2.3. Analyses

All analyses were carried out in the R statistical analysis software core v.3.5.0 using the RStudio v.1.1.447 interface (RStudio Team 2020; R Core Team 2020).

2.3.1. Modelling

The data were analysed by fitting both generalised linear models (GLMs) and generalised linear mixed models (GLMMs) to account for possible random treatment effects. GLMs were fitted using the base function *glm*, while GLMMs were fitted using functions in the package *lme4* (Bates et al., 2020). For both models, temperature was treated as a categorical variable, given the expected non-monotonic thermal response of the flies. Founding population size and number of introduction events were treated as continuous variables. For the GLMM, treatment batch was added as a random effect, in case batches that were started on different days influenced the outcome. Models were fitted with full interactions between terms: outcome ~ temperature * population size * introduction events and then simplified where possible (Crawley, 2013). Outcome was initially modelled as a binomial response variable with a logit link function. If GLMs were over-dispersed, a quasibinomial response variable was instead adopted (Crawley, 2013).

Relative model performance was evaluated by comparing the Akaike information criterion (AIC) or quasi-AIC (QAIC) values between models for under- or over-dispersed models, respectively (Bolker et al., 2009). AIC values could be extracted directly, while QAIC values were calculated using the package *MuMIn* (Barton, 2020) in R.

As temperature is very likely to have a large effect on reproduction, growth and establishment in an insect, it may obscure the effect of other factors. To determine the effect of founding population size and number of events within each environment, GLMs and GLMMs were also fitted to outcomes within each temperature treatment and compared using AIC or QAIC values as above.

The best-fitting models were further bootstrapped to increase robustness and determine 95% bias-correct, accelerated (BCa) confidence intervals. For GLMs, this was performed using the *Boot* and *Confint* functions within the *car* package (Fox and Weisberg, 2018; Fox and Weisberg, 2019) using 1000 iterations. For GLMMs, the function was more computationally intense and therefore 500 iterations were performed using the *bootMer* and *confint.merMod* functions in the package *MuMIn* (Barton, 2020).

2.3.2. Effect sizes

The above models are able to show which variables and interactions are statistically significant, but do not show the size of the effect of each one. Thus it is difficult to determine if a statistically significant variable or interaction is biologically relevant. For this, it is more useful to calculate effect sizes with bootstrapped confidence intervals (Cumming, 2014). Bootstrapped confidence intervals also give an indication of power of the test, i.e. the maximum effect size that our test can detect (Colegrave and Ruxton, 2003).

Effect sizes were calculated first as unstandardised, as is recommended to allow future inclusion in meta-analyses (Cumming, 2014). This was done using the function *bootES* in the package of the same name (Gerlanc and Kirby, 2015) with 5000 iterations. When contrasting the effect of two conditions, a weight of −1 and +1 was assigned to each by default (Kirby and Gerlanc, 2017). When comparing multiple conditions to find the global effect, contrast weights were calculated as described in Kirby and Gerlanc (Kirby and Gerlanc, 2013).

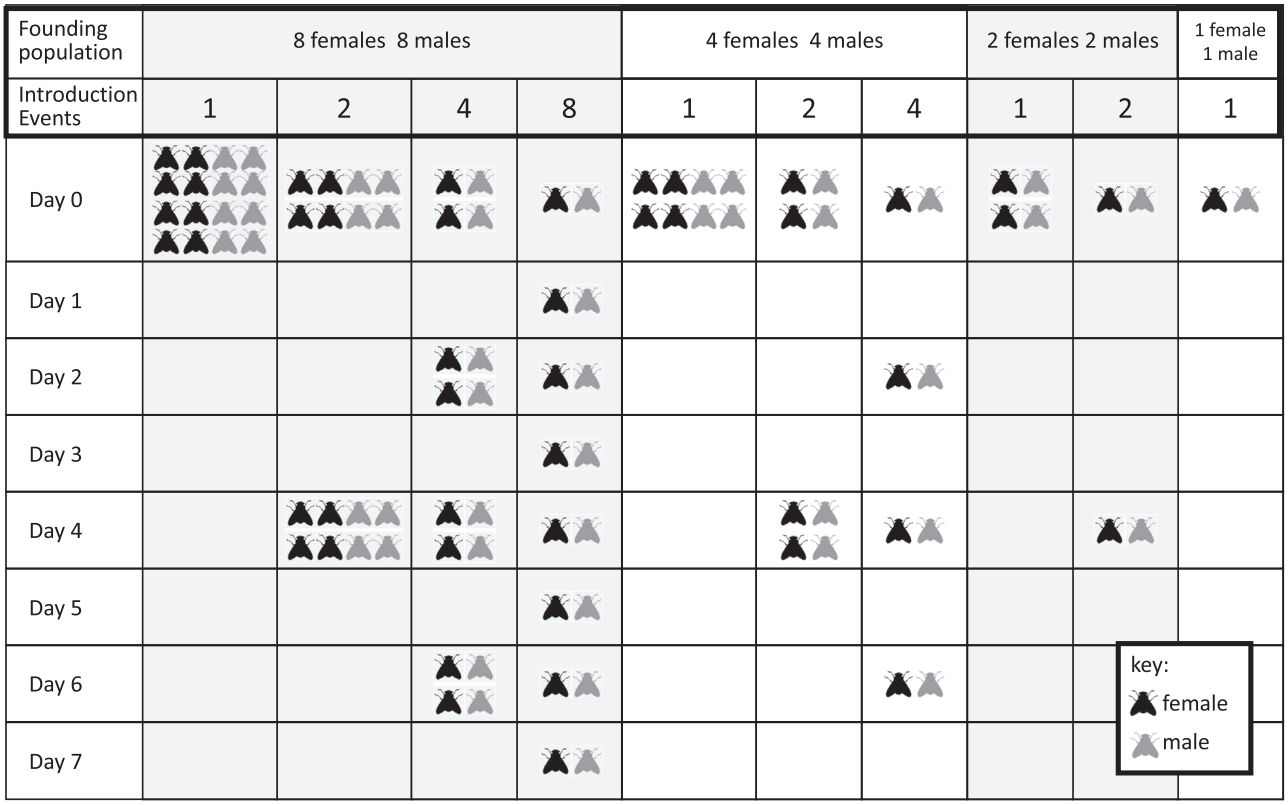

Fig. 1. Founding population sizes and number of introduction events of adult *Drosophila melanogaster* used to test establishment success of colonies. Treatment containers were wide-mouthed glass jars of 1 L, 500 mL, 250 mL and 125 mL with 160 mL, 80 mL, 40 mL and 20 mL diet media for founding population sizes of 16, 8, 4 and 2 adult flies, respectively. All treatments were repeated at 13 °C, 22 °C and 32 °C and replicated ten times.

Thereafter, Hedge's *g* was calculated as an unbiased, standardised estimate of effect size in a similar manner, also bootstrapped with 5000 iterations (Kirby and Gerlanc, 2017; Kirby and Gerlanc, 2013). Hedge's *g* is more robust to small sample sizes (Kirby and Gerlanc, 2013), but does assume a normal distribution of data (Algina et al., 2006). However, by calculating bootstrapped BCa confidence intervals along with Hedge's *g*, this metric performs well even if the assumption of normality is violated (Algina et al., 2006; Kelley, 2005).

Results from the models were extracted, collated into data frames and visualised using the *ggplot2* package (Wickham, 2016) in R.

3. Results

3.1. Overall establishment and power of experiment

Colony establishment success was 70–100% at the optimal rearing temperature (22 °C), 0–90% at 13 °C and 0–80% at 32 °C (Table 1). As expected, colony establishment was far more variable at the thermal extremes than at the optimal temperature.

3.2. Modelling

3.2.1. GLMs

The GLM containing all variables and all interactions fitted to the data the best (supplementary material). Simplifying the model by removing interactions or variables did not lower the AIC and in some cases resulted in over-dispersed models. However, in this overall model no term was significant and most of the confidence intervals were large (Fig. 2). Thus, although it is the best model in this context, it does not seem to sufficiently describe the data to give confidence in the results.

Table 1
The effect of varying the number of introduction events and the number of individuals per introduction event on the establishment success of *Drosophila melanogaster* colonies ($n = 10$ for each treatment). See Fig. 1 for a graphical representation of the experimental set-up.

Founding population size (1:1 female:male)	Number of introduction events	Number of colonies established ($n = 10$)		
		13 °C	22 °C	32 °C
16	1	8	10	5
16	2	2	10	6
16	4	7	10	8
16	8	9	10	2
8	1	4	7	5
8	2	5	10	4
8	4	1	10	5
4	1	4	10	3
4	2	1	9	6
2	1	0	9	0

GLMs within each temperature showed different influences of variables at different temperatures. At the thermal extremes (32 °C and 13 °C), increasing founding population size significantly increased the probability of establishment, while at the optimal temperature (22 °C), neither aspect of propagule pressure had a significant influence. Additionally, the GLM at 22 °C did not perform well because seven of the ten replicated trials had 100% success rate. Confidence intervals for both variables were consequently large for the 22 °C GLM (Fig. 2) (note the larger axis scale relative to that for 32 °C and 13 °C). The number of introduction events did not have a significant influence on establishment success in any of the models, possibly because it is correlated to found-

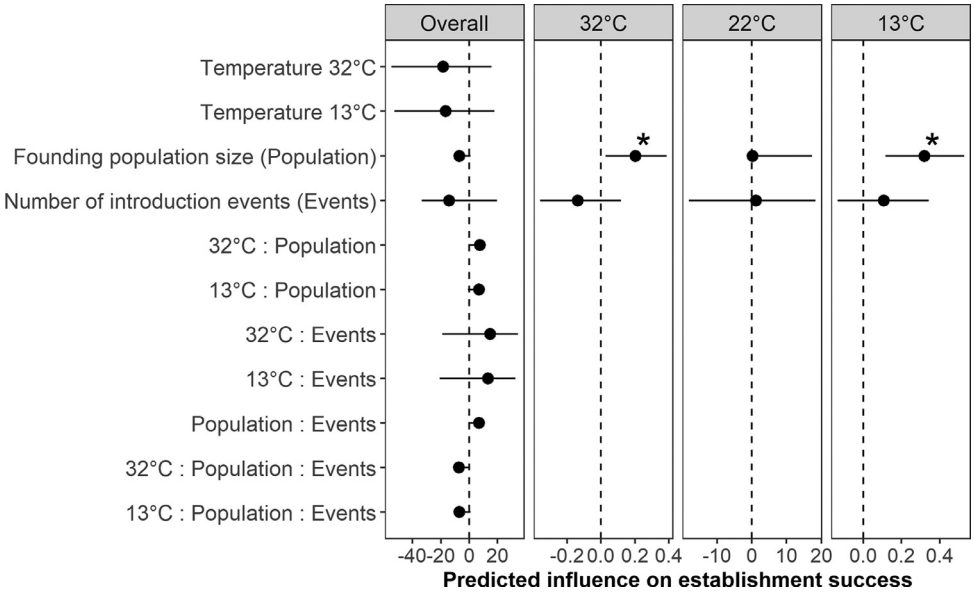

Fig. 2. The relative influence of temperature, founding population size, number of introduction events and interactions of these on the establishment success of *Drosophila melanogaster* colonies, modelled using bootstrapped generalised linear models (GLMs). Establishment success here is defined as offspring surviving to adulthood. Separate GLMs for the experiment overall and within each temperature treatment were produced and are graphed separately in each panel (left to right, note the different axis scales). Dots indicate the coefficient sizes estimated by the GLMs and the lines show 95% confidence intervals. Coefficients signify the size and direction of the influence of each of the predictors on establishment success, with positive values indicating a higher establishment success. A dashed line at zero is included to facilitate interpretation of coefficient values and confidence intervals. Confidence intervals which do not include zero indicate that the predictor has a significant influence and are indicated by an asterisk (*).

ing population size. This idea was explored further when testing effect sizes (below).

3.2.2. GLMMs

Most of the fitted GLMMs did not perform well (supplementary material), with four of the five models which included all terms failing to converge. Simplifying the model by removing terms did not lower the AIC. Thus we chose to retain the model with the lowest AIC that did converge, which contained all variables but no interaction terms. Bootstrapping this model resulted in 498 bootstrapped values, as two runs failed.

GLMMs fitted within each temperature performed even more poorly than the overall ones. Many of the models resulted in a singular fit, meaning that the model fit was on the boundary of feasible parameters (Bates et al., 2020). Bootstrapping these models did not improve results as many bootstrap iterations failed, model fits were singular or models did not converge. We thus did not examine the GLMMs further. Full details of the GLMM outcomes can be found in the supplementary material.

3.3. Effect sizes

Calculation of effect sizes enabled us to detect and visualise biologically relevant information far more easily than GLMs or GLMMs had done (Fig. 3). Effect sizes are of necessity calculated as pair-wise comparisons (weighted, if a global effect is desired) and thus can give more information about each aspect of the experiment in addition to the global effect.

As expected, temperature had a large and significant effect on establishment success: when moving away from optimal, establishment probability declined by 51% (95% CI [41–61%]) and 54% (95% CI [42–64%]) at hot and cold thermal extremes, respectively. The apparently small global effect of temperature is just a result of the strong non-linear relationship, where establishment peaks at optimal but declines to either extreme.

Overall, both founding population size and number of events increased establishment success. Founding population size had a greater and significant positive effect on establishment success (Fig. 3). On average, the global effect of doubling the founding population was to increase the chance of establishment by 25%. It is interesting to note that the effect size was not consistent for changes in different founding population sizes or number of introduction events. For instance, doubling of founding population from 2 to 4 had a larger effect than doubling it from 4 to 8 individuals.

Within each temperature, the effects of increasing founding population size and number of introduction events were more noticeable at the thermal extremes (32 °C and 13 °C) and negligible at optimum (22 °C) (Fig. 3). The effect of increasing founding population size was to increase establishment at both 32 °C and 13 °C, although far more strongly at 13 °C (effect size at 32 °$C = 0.21$; at 13 °$C = 0.46$; Fig. 3). Similarly, increasing the number of introduction events had a small non-significant effect at 32 °C, while at 13 °C it increased establishment significantly (effect size at 32 °C = −0.017; at 13 °$C = 0.36$; Fig. 3).

There was likely to be a strong correlation between founding population size and number of introduction events, as bigger founding populations could accommodate more events. Thus the increased probability of establishment with a higher number of introduction events could simply be due to larger founding population size. To test this, we controlled for founding population size by calculating the effect of number of introduction events at a single large founding population size (Fig. 3, last line). This showed almost no global effect (effect size = 0.0056) but did show more marked effects within the hot and cold thermal extremes. Interestingly, the effect at 32 °C was to decrease establishment success (effect size = −0.31) while at 13 °C increasing number of events increased establishment success (effect size = 0.32).

Direction and (corrected) sizes for Hedges *g* were very similar to those obtained from the unstandardised effect size calculations and were thus not graphed (supplementary material). As for the unstandardised effect sizes, Hedge's *g* showed a far greater effect of temperature (Hedges $g = -1.3$ and −1.4 for 32 °C and 13 °C, respectively) than for founding population size (Hedges $g = 0.52$ overall) or number of events (Hedges $g = 0.29$ overall) on population establishment.

4. Discussion

Our laboratory study corroborates previous observational, experimental and theoretical studies that have found establishment success is determined by an interaction between environmental conditions, founding population size and the number of introduction events (Duncan et al., 2014; Duncan, 2016; Bacon et al., 2014; Yeates et al., 2012; Vahsen et al., 2018; Hedge et al., 2012; Sinclair and Arnott, 2016; Sinclair and Arnott, 2017).

In this study, the effect of temperature was large and obvious, as might be expected given the broad range tested, and initially masked

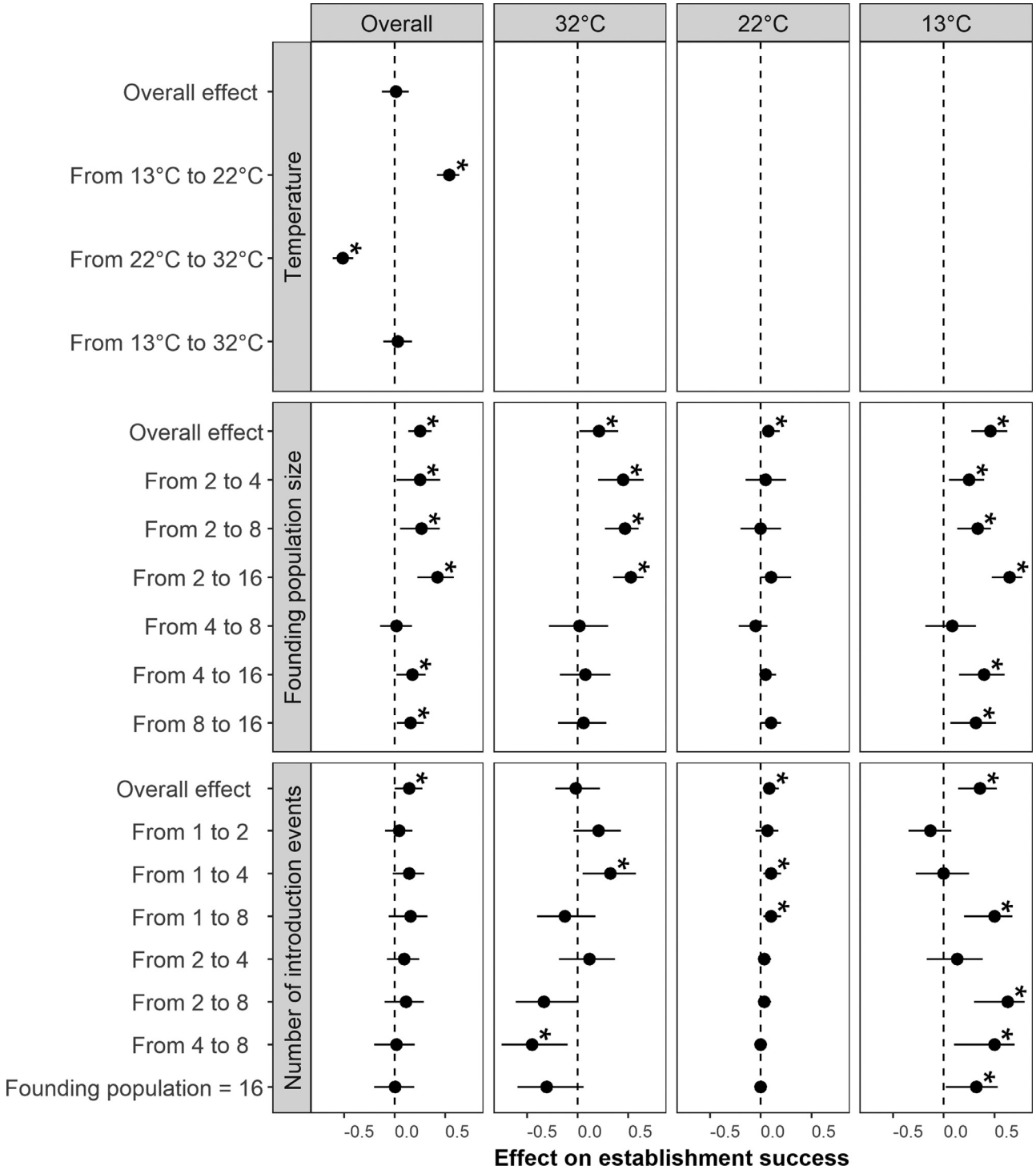

Fig. 3. The effect of temperature (top), founding population size (middle) and number of introduction events (bottom) on establishment success of *Drosophila melanogaster* colonies. Bootstrapped unstandardised effect sizes for the experiment overall and within each temperature treatment were calculated and are graphed separately in each panel (left to right). The unstandardised effect size (dot) and 95% confidence interval (line) are graphed for each global or pair-wise comparison shown on the vertical axis. A positive effect size denotes an increase in establishment success as the variable shifts from a to b (as stated on the vertical axis). A dashed line at zero is included to facilitate interpretation of effect size values. Unstandardised effect sizes can be interpreted in the original units of measurement (i.e. temperature in ˚C, founding population size in number of individuals and number of introduction events). Confidence intervals which do not include zero indicate that the effect is significant and are indicated by an asterisk (*).

the comparatively smaller role of propagule pressure. This confirms our first prediction that temperature would have the greatest effect on establishment. Given this, it may have been tempting to conclude that propagule pressure played a very small role in establishment success. However, when viewed within each controlled temperature, the role of propagule pressure became apparent even within the confines of this fairly limited laboratory experiment.

We expected propagule pressure in this study (both in total founding population size and in number of introduction events) to influence establishment under all conditions, with a greater effect at thermal extremes of viable population growth. This prediction was only partially confirmed: the effect of propagule pressure was negligent at optimal temperature, but became apparent at the thermal extremes. This corroborates theory, as the effects of demographic stochasticity are more likely to have deleterious effects in adverse environments as individual differences in fitness and survival are amplified at low population sizes (Fauvergue et al., 2012; Duncan et al., 2014; Bajeux et al., 2019).

Interestingly, increasing the number of introduction events had different magnitude and directions (i.e. sign) of effects at 13 °C and at 32 °C, thus disproving our final prediction. This context-dependence is likely due to different physiological mechanisms at play at the two thermal extremes and the generally asymmetric nature of thermal performance curves in ectotherms, as has been shown previously (Sinclair et al., 2016; Buckley et al., 2017; Frazier et al., 2006).

Low temperatures slow insect metabolism and development and suppresses activity, thus, slowing population growth rates and reducing fitness. However, at sub-lethal cold temperatures such as those tested here very little direct physiological harm is caused to the insect itself. Thus, the low temperature population establishment scenario can be overcome by adding more individuals to the population to facilitate population growth, albeit slow. This would be especially useful if the new individuals were sourced from optimal environments, where their own metabolism, reproductive capacity and likely body condition were already maximised. The positive effect of multiple introduction events is also likely to be seen in the case where Allee effects are not acting strongly on the population and small introductions are not likely to go extinct before the addition of more individuals (Sinclair and Arnott, 2016). Indeed, this is exactly what was seen in the current study at 13 °C, where larger founding population sizes and increased numbers of events (i.e. adding individuals in increments rather than all at once) had a marked positive effect on establishment success. Thus we can postulate that at the low temperature tested here, Allee effects were minimal and that the strongest driver of successful establishment was the repeated introduction of optimal individuals to build up a population.

Conversely, high temperatures are far more detrimental to the insect and set a harder boundary on population establishment than cooler temperatures and may cause sterility and eventual death, even at temperatures which are not immediately lethal (Walsh et al., 2019; Araújo et al., 2013). Both the detrimental physiological effects and increased metabolic rate at this high temperature may contribute to demographic stochasticity playing a far larger role than at cold temperatures. In the current study, this can be seen by the very small effect of increasing founding population size at 32 °C. Adding more individuals would not compensate for physiological damage and so, while it dampens the effects of demographic stochasticity, it does not completely overcome it. Similarly, increasing the number of events had very little effect, as small introduction events are more likely to go extinct (or at least become performance impaired) before the next introduction event, resulting in very little positive effect from new additions.

The fact that the number of introduction events did not make a difference to global establishment success may be because it was confounded with effects due to temperature and founding population size. When tested in isolation at the largest founding population size, the number of introduction events did have an effect at the thermal extremes (Fig. 3), although this was not as large as that due to founding population size itself. There are several reasons why the number of events may not have had a large effect in this study. *Drosophila melanogaster's* egg to adult life cycle is fairly short compared to the adult longevity and multiple generations are expected to overlap. In this experiment, introduction events were spaced out over eight days. This was chosen as at 32 °C emergence of the new generation of adults could be observed as from day nine and thus eight days was the maximum available time for introductions. However, due to adult longevity, introductions on this time scale may have been functionally indistinguishable from a single introduction event. This possibility seems plausible, given that at low temperature (where the life cycle is slowest), the effect of multiple introductions was more marked and resulted in higher establishment success. It would be useful to further investigate this in *D. melanogaster*, possibly using a low temperature (to maximise experimental time) and varying the timing of introduction events. Introduction events could also foreseeably be of different life stages in order to vary the potential overlap of reproductive stages. Moreover, one might expect that multiple introduction events have more value for establishment success in situations of environmental stochasticity. If conditions are not always suitable for population establishment (but still allow for population persistence), then multiple introduction events will be much more likely to coincide with an invasion window than a few introductions of large numbers (Fauvergue et al., 2012; Dressler et al., 2019). This is for instance true in seasonality, as introduced populations may be more likely to establish when climate conditions at the origin and introduced region match more closely (Tatem and Hay, 2007; Hill et al., 2017).

5. Conclusion

Abiotic factors, biotic factors and introduction dynamics interact to determine whether a population will establish in a new environment. For *Drosophila melanogaster* in our mesocosm experiments, temperature (an important abiotic factor determining survival and growth rates) was most important in determining successful establishment of colonies in the laboratory. In this experiment, a larger founding population increased the chances of establishment in an inhospitable environment (i.e. at the thermal extremes), while at optimal temperature, measures of propagule pressure had no influence. The relative influences of founding population size and number of events had different effects at hot and cold thermal extremes, highlighting the different physiological and stochastic mechanisms at play.

In general, introduction of more individuals increases the chances of at least one individual surviving to reproduction. This difference becomes increasingly important when faced with harsh or unsuitable conditions and could manifest differently for diverse species with more complex life-histories and evolutionary adaptations to climate (Iles et al., 2016; Fauvergue et al., 2012; Bajeux et al., 2019; Yeates et al., 2012; Buckley et al., 2017).

Authors' contributions

DS designed the study, carried out the experimental work, collected data and performed statistical analysis and drafted the manuscript. JW participated in the design of the study, assisted with data and statistical analysis, contributed to writing and critically revised the manuscript. JST conceived the study, participated in the design of the study, assisted with data analysis and interpretation, contributed to writing and critically revised the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cris.2021.100011.

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